

Shouldering the burden

A new approach to shoulder pain

The work of Professor Richard Page and Dr Stephen Gill, the Barwon Centre for Orthopaedic Research and Education (B-CORE), uses sophisticated and modern technologies to examine gene expression and molecular pathways in painful conditions of the shoulder, including frozen shoulder. Ultimately, they hope to improve quality of life and treatment outcomes for people of all ages suffering from musculoskeletal pain such as shoulder pain from tendon disease or osteoarthritis. In addition, Professor Page and Dr Gill use biobanking to answer questions regarding the cause, mechanism and progression of musculoskeletal conditions.

Painful musculoskeletal conditions are highly prevalent in the community and are associated with significant disability and health burden. Conditions affecting the shoulder have a high prevalence and impact on a wide range of age groups and common activities, with up to one in twenty patients presenting to their GP with shoulder pain.

Adhesive capsulitis (AC), or frozen shoulder, is just one of a range of common conditions affecting the shoulder and its prevalence in the general population is around 2%. The disorder presents as stiffness, pain and dysfunction of the ball and socket joint of the shoulder called the glenohumeral joint. It is often accompanied by issues with the surrounding rotator cuff muscles. AC is most common in women and those aged 40-60. While AC is often self-limiting and may resolve within two years in some people, it can cause ongoing problems for many individuals, particularly in diabetics.

Although much is known about how AC presents, less is known about the underlying causes of the condition, and it is this that Professor Richard Page, with collaborator Dr Stephen Gill, hopes to learn more about. Adhesive capsulitis is associated with rotator cuff disease, a number of other endocrinopathies and trauma, suggesting a metabolic or inflammatory common pathway in the pathogenesis. Indeed, it has been suggested that secondary AC may be associated with a history of trauma or surgery, heart attack or diabetes. The prevalence in diabetes has been cited as high as

38.6% and AC is the most common joint condition associated with diabetes.

THE AdCaB STUDY

A recent paper published by Professor Page presented the protocol for the adhesive capsulitis biomarker (AdCaB) study which will investigate gene expression alterations in people with primary and secondary (due to indirect causes) AC compared to controls. While the control subjects will not have AC, they will suffer from shoulder instability which does not have the same associated inflammation.

The prospective, longitudinal, multi-centre study will be conducted at two large regional hospitals in Victoria, Australia and the researchers aimed to recruit a minimum of 25 participants per group. In order to be part of the study, subjects will meet a strict set of criteria.

TRANSCRIPTOMICS

Professor Page's work includes looking at transcriptome-wide alterations in gene expression in the shoulder joints of people with AC compared to those who have shoulder problems, but lack inflammation. The transcriptome represents the first step in gene expression and involves copying the DNA of a particular gene to make an RNA molecule. By examining which genes are being transcribed, we get a picture of which genes are most active under specific conditions. For example, although we have genes for many different hair colours, colour is determined by which genes are selectively transcribed and expressed in that individual.

Previous research has found that there may be a genetic predisposition to developing AC. Therefore, the work of the team at B-CORE and Geelong Orthopaedics might help understand



Professor Page will examine blood and urine samples in the search for biomarkers.

how gene expression influences AC. The AdCaB protocol paper is the starting point for a suite of studies looking at musculoskeletal gene expression.

BIOMARKERS

The second part of Professor Page's AdCaB study protocol is to search for serum and urine biomarkers to help further understand the diagnosis and stage of AC. Biomarkers, or biological markers, consistently appear in particular circumstances, such as a specific disease or physiological process.

In order to do this, blood and urine samples will be collected before subjects undergo surgery, and tissue samples will be collected during the operation. Total RNA will be extracted from the samples and genome sequencing technology will be used to determine which genes are being expressed. Once genes have been identified, the team will conduct other analyses, such as pathway analysis, which explores the biological pathways in which the genes are involved.

Furthermore, in order to identify potential biomarkers, computational techniques will be used to identify which genes are most commonly seen in AC patients. Professor Page hopes that this will allow characterisation of groups of genes which could guide diagnosis of AC for future patients, as well as shedding light on the underlying mechanisms of disease.

Understanding disease pathways represents an important step towards developing therapeutics which target these mechanisms.

CLINICAL OUTCOMES

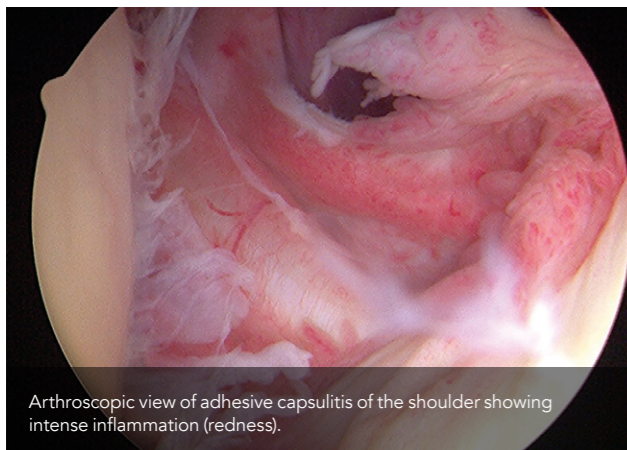
The third part of the AdCaB study will explore joint surgery outcomes in people with AC. Patient-reported outcome measures will be collected before surgery, and

at three and 12 months after surgery. The measures to be collected by the team at B-CORE and Geelong Orthopaedics will include the Oxford Shoulder Score and the Oxford Shoulder Instability Score. The pre and post-operative data will be analysed with statistical software, taking into account factors such as age and whether participants are right or left-handed, as these may influence results.

Painful musculoskeletal conditions, such as those affecting the shoulder are highly prevalent in the community and are associated with significant disability and health burden.



An X-ray of a woman suffering from adhesive capsulitis.



Arthroscopic view of adhesive capsulitis of the shoulder showing intense inflammation (redness).



Arthroscopic view of early arthritis of the shoulder – with irregularity of the cartilage joint surface.

BIOBANKING

The broader aim of Professor Page's work is to improve the diagnosis and treatment of conditions affecting the shoulder, as well as other related musculoskeletal conditions. One way that Professor Page is doing this is through linking the research data to the Barwon Joint Registry in

management of challenging joint replacement infections. The researchers are using this tissue banking platform to investigate a range of conditions from osteoarthritis, joint instability, tendon and rotator cuff disease, frozen shoulder and joint replacement infections. As well as exploring the conditions themselves, Professor Page

about transcriptome expression and molecular pathways underlying these disease processes. In doing so, the researchers hope to identify novel biomarkers, leading to earlier diagnosis and optimisation of treatment options for a range of musculoskeletal conditions, including frozen shoulder and shoulder arthritis.

PRELIMINARY RESULTS

Preliminary results of the AdCaB study show that the group at B-CORE and Geelong Orthopaedics were able to recruit 22 patients to the AC group and 26 patients to the control group. They were able to identify 545 genes which were differentially expressed in AC, either significantly up or down-regulated compared to controls. As expected, the genes which were up-regulated included those linked to inflammatory processes and collagen and bone cell synthesis. However, the group did identify some novel genes which had not previously been associated with AC, including potential vitamin C-dependent pathways. Analyses are still ongoing, with the next steps involving processing the serum and urine samples, as well as doing sub-analyses for risk groups such as age, gender and those with diabetes.

Linking this work to the rotator cuff and shoulder osteoarthritis research being done by Professor Page, Dr Gill and colleagues will provide new information about the pathogenesis (mechanism of disease), diagnosis and staging of AC, as well as evaluating clinical outcomes of people who undergo shoulder surgery. In addition, screening for diagnostic biomarkers will lead to a more directed and timely treatment for sufferers of this debilitating condition.

Australia, a database for evaluating outcomes of joint replacement surgery, to enable the follow up of patient treatment outcomes.

As part of this work, tissue and blood samples are being collected from joint replacement infections with the aim to improve early diagnosis of infections for future patients. This biobanking work may also yield potential biomarkers that can improve the diagnosis and

is also interested in the links to systemic metabolic conditions, such as diabetes, which affect patients.

These studies recruit from a surgical cohort and involve the collection of tissue normally excised and discarded at surgery, as well as blood and urine samples. Collecting the samples during surgery eliminates additional injury or discomfort to participants. These studies will increase knowledge



Shoulder pain can be very debilitating and places a large health burden on the community.

Behind the Research



Richard Page

E: Richard.page@deakin.edu.au
T: +61 3 5215 0900
@BCORE5

W: <http://bcore.deakin.edu.au>

W: <https://www.deakin.edu.au/about-deakin/people/richard-page>

W: <http://geelongorthopaedics.surgery/prof-richard-page>



Stephen Gill

E: Stephen.gill2@deakin.edu.au

W: <https://www.deakin.edu.au/about-deakin/people/stephen-gill>

Research Objectives

Professor Richard Page and Dr Stephen Gill use a new approach to better understand musculoskeletal conditions.

Detail

Barwon Centre for Orthopaedic Research and Education
Education and Research Centre
St John of God Hospital Geelong, Victoria, Australia

Bio

Richard Page is an orthopaedic shoulder surgeon with over 20 years of research and surgical education experience. He is the Founding Director of B-CORE, St John of God and has been Barwon Health Chair of Orthopaedics at Deakin University since 2014. His interests include outcomes of shoulder and upper limb surgery, osteoarthritis and joint replacement surgery, biomechanics and related trauma topics.

Stephen Gill is a clinician-researcher who assists Professor Page to develop and implement B-CORE's research programs. Since completing his PhD in 2009, Stephen has completed a broad range of health services and musculoskeletal research. He continues to work as a senior clinician physiotherapist.

Funding

- Barwon Health – Education, Training and Research Profile Fund
- Australian Orthopaedic Association Research Foundation

Collaborators

- **Deakin University - Centre for Molecular and Medical Research**
<https://www.deakin.edu.au/cmmr/our-research/metabolic-and-musculoskeletal-medicine-research>
- **St John of God Hospital Geelong**
<https://www.sjog.org.au/our-locations/st-john-of-god-geelong-hospital>
- **Barwon Health**
<https://www.barwonhealth.org.au>
- **Geelong Orthopaedics**
<https://www.geelongorthopaedicsgroup.com.au>

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Personal Response

Do you have any new results from the AdCaB study which you are able to share with us?

// We compared the genes from the tissue of patients with adhesive capsulitis (AC) with those having surgery for shoulder instability to determine potential biomarkers specific to AC. Tissue samples were collected during surgery, the RNA was extracted, and RNA-sequencing-based transcriptomics undertaken (using Next Gen Sequencing). We then analysed the results to identify biological processes and pathways that were active. When we spotted a number of genes that seemed to be differentially expressed, these were validated using a process known as real time RT-PCR, which amplifies those genes.

The results showed that patients with AC had increased expression of three genes (PDGFB, COL18A1 and MMP9) and reduced expression of a fourth (TNFA). This analysis has identified interesting and novel pathways to enhance our understanding of AC and provided potential future targets for staging and treatment of this painful and debilitating shoulder condition. **//**